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Soobschenie I

DRY LIVING TULAREMIA VACCINE OF NIIEG OF THE
RED ARMY
Report I

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(In Russian)

DRY LIVING TULAREMIA VACCINE OF NIIEG OF THE RED ARMY
Report I

Experimental investigations published in the Soviet and in the foreign press verify the fact that tularemia vaccines derived from dead microbes cannot immunize animals (white mice and guinea pigs) highly susceptible to tularemia, regardless of the method of their preparation and despite a triple vaccination; such vaccines raise but slightly the animal resistance, while vaccines derived from living diluted tularemia microbes safeguard these animals after a single injection against subsequent inoculation with massive doses of virulent culture.

Of the work indicated, much attention is due the investigations of our native authors (El'bert and Gaiskii, 1935). On the basis of data obtained by study of the immunity to tularemia, Gaiskii recently (1944) proposed for the vaccination of man two diluted strains, Ondatra IV and No. 15 (according to professor Khatenever's report, Gaiskii had obtained the latter strain from him with an already attenuated virulence). The investigations of Gaiskii have demonstrated that vaccine from the indicated strains possesses highly immunogenic, antigenic and allergenic properties. Tests made of this vaccine by Gaiskii on man in tularemia nidi (6652 humans were vaccinated) and by Kosmachevskii under clinical conditions have shown that this vaccine is harmless and causes to but a small degree a local and in rare cases a general

reaction with a slight increase in temperature.

Regardless of the fact that tularemia vaccine obtained from living attenuated vaccine microbes possesses an undisputed advantage over vaccines derived from dead microbes, its widespread application (Similar to that of any other living vaccine) encounters serious obstacles as a result of its instability when stores; in but a few hours of storage at room temperature, or in 12-15 days of storage at refrigerator temperature, this vaccine loses its effectiveness. The circumstance indicated compels it that living vaccine be cultivated at the place where the vaccination is performed, and this calls for the importation of a qualified bacteriologist with a small laboratory. Considering the complexity of growing tularemia culture and the necessity of storing vaccine strains under fixed conditions, one must admit that it is practically impossible to organize the production of living vaccine on the spot or in separate cases.

Thus it became urgently necessary to develop a highly effective vaccine preparation from living cells, capable of preserving its immunogenic properties for an extended time.

To develop a preparation capable of satisfying the demands indicated, we u utilized the above described strains of Ondatra IV and No. 15 obtained from Gaiskii. We used the method of drying in a high vacuum the frozen suspension of microbes of dry vaccine from the indicated strains in a special medium.

Investigations of fellows of the Scientific Research Institute of Epidemiology and Hygiene of the Red Army (Faibich, Del'nik and Zagor, 1940, and again Faibich and Karnesv, 1942) have shown that, under conditions of drying microbe cultures in appropriate media (media of drying), this process of drying, suggested by Shekkel in 1909, permits the production of dry preparations capable of retaining their properties unchanged for a long time. (begin p. 60). Media best adapted for this purpose proved to be those containing saccharose or other carbohydrates (Maltose and lactose) in amountsof 10-15%, with an added 1.25-1.5% of gelatin for the

purpose of improving drying conditions (Faibich and Karneev, 1944). Our experiments have shown that the best media for drying living tularemia vaccine were saccharose-agar-gelatin (the latter serves concurrently as a growth culture).

Drying of living tularemia vaccine was carried out in an apparatus consisting of diverse equipment developed for vaccine drying by fellows of NIIEG (Karneev, Del'nik, Grudenko and Chernykh), and of a high vacuum pump of the Stokes Firm (model 412 and 212) that permits the evaporation of water steam into atmosphere without the use of chemical absorbents or low temperature for humidity condensation.

The intracacy of preparing media for growing causal agents of tularemia known at the present time (egg yolk, MacCoy, (Mak Koi) and cyctine-blood agar of Francis (Francis) and the impossibility of their use in mass production of bacterial preparations has compelled many authors to search for simpler media. Thus, Bereninova established (1938) the feasibility of cultivating tularemia microbes in hepatic liquid media with protective colloids; Olli and Mikhailova (1944) have cultivated tularemia cultures successfully on yeast media. Tamura and Dzhaibbi (1943) were successful in cultivating the tularemia agent in liquid media containing hydrochloric acid hydrolysate of gelatin or casein and extracts of liver and blood cells. Gibby

The experiments of the authors referred to and our own numerous investigations enabled us to propose the following media for mass production of tularemic bacteria preparations: 1(semi-liquid agar consisting of pancreatin hydrolyses of liver and gelatin, saccharose, white gelatin, starch, agar and water; 2) a thick agar medium in two variants, the first of which contains pancreatin hydrolyses of liver, blood and gelatin, milk serum, cystine, starch, table salt, agar and water, and the second - pancreatin hydrolysis of blood, autolysis of yeasts, milk serum, cystine, starch, table salt, glucose, agar and water.

For the purpose of preserving in the media the largest possible amount of vitamins needed for the cultivation of the causal agent of tularemia, the hydrolyses of liver and blood, contrary to the generally accepted method, were prepared without

a preliminary thermal treatment, i.e., from raw material. For the precipitation of yolks and other substances which retard microbe growth, all hydrolyses were treated with sulfuric acid aluminum. In the growth media was established a slightly alkaline reaction (pH = 7.1 - 7.2), and in drying media - a neutral one.

The proposed media are sterilized in autoclave at a temperature of 120° C. If specific rules are observed in dispensing, then these media produce a high yield microbes (on the average, on 1 cm 3 of medium no less than 1.5 mlrd. (billion) from a semiliquid and 3 mlrd. from a thick agar one): as regards their properties, tularemia cultures grown on them do not differ from the cultures obtained from egg yolk media.

The media indicated were used for the preparation of dry living vaccine of two variants: from cultures on a semiliquid medium (containing 4% of saccarose instead of salts) and from culture derived from a thick agar medium. In order to obtain dry vaccine of the first variant, an additional solution of saccharose up to 10% was added to a 2-3 full-day old culture on a semiliquid medium, after which it was was stirred and poured in ampules at 1cm^3 each and then dried. (begin p.61). As a result the semiliquid medium was simultaneously a medium for growth and a medium for drying. At the beginning this semiliquid medium was prepared without saccharose, but with an addition of 0.85% of table salt. It proved subsequently that dry vaccine prepared from cultures (grown) on such a medium contained but a small amount of living microbes and quickly lost its effectiveness when stored. Therefore table salt was substituted with saccharose (4%). To prepare dry vaccine of the second variant, the cultures on a thick agar medium, following 2-3 full days of growth at 37° C, were washed with a drying medium, examined as to purity, diluted with the same drying medium up to a titre of 2-5 mlrd. microbes on 1 cm^3 and were poured in ampules at 1 cm^3 each. Before drying, the vaccine poured into ampules was being frozen in ice mixed with table salt; After their drying for a period of 14 to 18 hours, the ampules were disconnected so as to preserve high vacuum within them.

It is known that the immunogenic capacity of living vaccines depends on the number of viable microbes within them. The dying off of the latter leads first to a reduction in, and later to a complete loss of their immunogenic properties. Hency determination of the number of living microbes is a most important factor in judging the extent of fitness of any vaccine preparation. Unfortunately, it is impossible to determine the number of living microbes in tularemia dry living vaccine by the number of colonies on hard nutritive media. An idea concerning the number of viable microbes in dry living tularemia vaccine was formed by means of its biological titer, i.e., by the final dilution of the vaccine in a semiliquid hepatic medium at 37° C, produced growth within 8-10 full days. In addition, prior to its dilution in a semiliquid medium, the dry vaccine was first converted into a suspension by adding to it a physiological solution up to the original volume. Use of the indicated method to determine the number of viable specimens produced results which coincided with date obtained from investigations on animals.

The investigations conducted showed that the drying of cultures of vaccine strains suspended in media containing 10-15% of saccharose, 0.1% of agar and 1.3% of galatin, permits to obtain a dry preparation with a high content of living microbes capable of enduring prolonged storage. Thus, the biological titer of a suspension of living vaccine microbes suspended in a saccharose-agar-gelatin medium equaled 10^{-8} or 10^{-9} and it kept at the same high degree also after drying. In the course of a year's storage this dry saccharose-agar-gelatin vaccine in ampules with a vacuum at a temperature 2-4° C, its biological titer did not change. Preservation of the vaccine at a high temperature led to a decrease in the number of living microbes. The higher the storage temperature, the more intensive was microbe mortality. After days of storage of dry vaccine at 18° C or after 75-90 days of storage at 26° C, its biological titer was equal to 10^{-4} or 10^{-5}. Dry vaccine preserved under the conditions indicated in dosage of 12½ mln. (million) microbes (according to the standards of TSGHYK (Central State Scientific Control Institute)

caused immunity of high intensity in guinea pigs and white mice, ensuring 95-100% of survival after their inoculation with 1,000 Dlm of virulent culture.

It must be pointed out that drying cultures of vaccine strains in a saccharose-agar-gelatin or in a saccharose-gelatin medium produced in both cases dry vaccine containing an almost equal number of living microbes. The saccharose-gelatin dry vaccine, however, proved less stable during storage. Thus, after its 60 day preservation at 18°C, as many living microbes were found in saccharose-gelatin dry vaccine as in saccharose-agar-gelatin vaccine, preserved 250 days under the same conditions. (begin P.62) After 250 days of preservation at 18° C, saccharose-gelatin dry vaccine no longer produced growth.

Drying of suspension of living vaccine in other media without saccharose and agar led to intensified dying-off of living microbes in the process of drying as well as in storage in dry form even at refrigerator temperature. The biological titer of vaccine, dried only in the solution of gelatin, equaled first 10^{-3}, after 250 days of storage at 2-4° C it dropped to 10^{-2}, and after 60 days of storage at 18° C no living microbes were found in it whatsoever.

Thus, the results stated show that saccharose and agar possess protective properties, i. e., they increase the resistance of microbes in vaccine during drying from a frozen state, as well as during storage in a dry preparation; saccharose basically increases the resistance of living microbes during freezing and drying and partly during storage, while agar facilitates their longer preservation in a dry preparation. In drying media, gelatin exerts no notable influences on living microbes during the drying of vaccine and its preservation in dry form, it merely improves drying conditions and permits the drying of frozen suspensions containing a small number of microbes in 1 cm^3.

The protective action of carbohydrates during freezing was established by botanists as a fact long ago. Thus, Lidfors (1907) was the first to establish that egg yolks do not coagulate during freezing in a 5-10% carbohydrate solution. Later these date were confirmed by Maksimov, Okerman and Rikhter. In the investigations

of Okerman, Govorov and others, it was established that there is complete parallelism between the amount of sugar contained in the leaves of plants and their capacity to endure cold [1]. In our experiments it was also observed that there is a certain relation between the number of living microbes in dry vaccine and the part of saccharose in drying media. Thus, in vaccine that had been frozen in a medium with 10-15% of saccharose and then dried in a vaccum, were found more living microbes than in media containing 4-7.5% of saccharose. In the experiments of Lait, the preliminary treatment of spermatozoons of a frog with saccharose increased considerable their resistance in subsequent freezing. Many authors explain the protective role of carbohydrates in freezing of plants by their ability to fix a considerable amount of water, to reduce the freezing point and thereby to prevent the accumulation of ice crystals endangering the life of plants (Shmidt).

The mechanism of the protective action of saccharose in drying microbes by the "lyophilization" method is not known; it can only be presumed that saccharose, obviously, penetrates the microbe cell and fixes the amount of water needed to preserve its viability. As far as the protective action of agar is concerned, we are of the opinion, that its escence lies in its ability to envelop the microbes in the medium in which the vaccine is being dried and thereby to increase the protective function of the microbe capsules and to safeguard them against excessive dehydration.

Apart from this, there is some justification for the use of agar in media designated for drying living vaccine, because it has the capacity to increase the immunizing properties of microbe antigens, as established by Muromtsev.

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1. Citation concerns Shmidt, Anabioz. Biomedgiz, 1935.

The results of our investigations demonstrate also that the preservation of dry vaccine in ampules with a disturbed vacuum leads to a rapid dying-off of the microbes within it.

Thus, on the basis of attenuated tularemia strains, and by using the method of drying in a high vacuum from a frozen state in media containing saccharose (maltose and lactose), agar and gelatin, we obtained a stable vaccine preparation named "Dry Living Tularemia Vaccine of NIIEG". We have developed a method for its production, control and also conditions for this dry vaccine preparation, and have organized its mass production. The Dry Living Tularemia Vaccine of NIIEG has been tested on a large number of animals and man. The results of this work are described in a separate report.